Does Mitochondrial Genome Mutation in Subjects with Maternally Inherited Diabetes and Deafness Decrease Severity of Diabetic Retinopathy?

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Two types of retinopathy, diabetic and pigmentary, may be seen in subjects with maternal inheritance diabetes and deafness. The potential for interactions between the two retinopathies has not been explored. The mitochondrial mutation may affect development of diabetic retinopathy in subjects with MIDD by altering normal pathways of glucose metabolism. We identified five unrelated MIDD kindreds with 61 living maternal line family members. Twenty-three of the family members, 12 with diabetes mellitus and 11 without volunteered to be studied. Subjects were graded for severity of diabetic retinopathy and presence or absence of pigmentary retinopathy after slit lamp biomicroscopy, retinal photography of seven standard fields and fluorescein angiography. Blood was taken, in the fasted state, from MIDD subjects (duration of diabetes 17.0 ± 6.9 yr) and non-diabetic subjects with the mutation, for assay of sorbitol and glucose and values compared with diabetic and non-diabetic control subjects without the mutation. Diabetic retinopathy was absent in 9/12 subjects (75 %), with 3 having mild non-proliferative retinopathy. No one had cataract. Red blood cell sorbitol levels, adjusted for ambient blood glucose, were significantly lower in MIDD subjects compared with diabetic subjects (1.16 ± 0.5 cf. 2.03 ± 1.1, $\times 10^{-3}$ g mmol^{-1}, $p = 0.04$). Pigmentary retinopathy was present in 15 of 23 subjects, of whom 13 had some abnormality of glucose tolerance. Abnormal glucose tolerance was strongly associated with the development of pigmentary retinopathy (odds ratio 19.5, $p = 0.008$). In conclusion, there appears to be a decreased prevalence of diabetic retinopathy and cataract in MIDD, which we propose is due to reduced glucose metabolism by the polyol pathway. Abnormal glucose tolerance increases the clinical expression of pigmentary retinopathy in subjects with a mitochondrial genome mutation. A greater understanding of the metabolic effects of mitochondrial DNA mutations has the potential to give insight into the mechanisms of diabetic retinopathy and other complications of diabetes mellitus. © 1998 John Wiley & Sons, Ltd.

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Introduction

Maternally inherited diabetes and deafness (MIDD), due to the 3243 A-G tRNA$^{LEU(UUR)}$ point mutation (the 3243 mutation) of mitochondrial DNA[1,2] is thought to account for approximately 0.5–3 % of all cases of Type 2 diabetes mellitus.[3–5] Two types of retinopathy are seen in patients with diabetes due to the 3243 mutation: diabetic and pigmentary.[6] Previous studies in MIDD subjects have reported the presence or absence of diabetic retinopathy but ignored the presence of pigmentary retinopathy.[7–9] The prevalence and severity of diabetic retinopathy in subjects with MIDD has not previously been reported and the potential for interactions between the two retinopathies has not been explored.

We hypothesize that the mitochondrial mutation, by reducing ATP and free NAD$^+$ availability, may decrease metabolism of glucose by the polyol pathway, resulting in a decreased severity of diabetic retinopathy in subjects with MIDD. Hyperglycaemia, instead, may lead to excess lactate production within the retinal pigment epithelium (RPE) resulting in increased expression of pigmentary retinopathy. To address our hypotheses, we determined

Abbreviations: MIDD maternal inheritance diabetes and deafness, the 3243 mutation 3243 A-G tRNA$^{LEU(UUR)}$ point mutation of mitochondrial DNA, RPE retinal pigment epithelium, RBC red blood cell, DM diabetes mellitus, IGT impaired glucose tolerance, NGT normal glucose tolerance, WESDR Wisconsin Epidemiological Study of Diabetic Retinopathy, HbA$_{1c}$ glycosylated haemoglobin, OR odds ratio, CI 95 % confidence interval, NAD nicotinamide-adenine dinucleotide

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the prevalence and severity of both diabetic retinopathy and cataract in diabetic and non-diabetic subjects with the 3243 mutation, the prevalence of pigmentary retinopathy and the relation of these prevalences to age, as ageing is thought to influence the clinical expression of mitochondrial mutations.[12] Red blood cell (RBC) sorbitol levels were measured, as a surrogate for tissue sorbitol,[10,11] to look for evidence of reduced polyol pathway activity in subjects with MIDD.

Patients and Methods

The study was conducted in accordance with the principles expressed in the 'Declaration of Helsinki'.

Patients

Twenty-three maternal line family members (12 M, 11 F) from five kindreds (Table 1) out of a total of 61 living maternal line family members at the time of the study, volunteered for retinal examination. Two of the five kindreds have previously been reported.[13,14] Of the 61 members, 18 had diabetes mellitus (DM, 30 %) and 43 were non-diabetic (70 %). Twelve of 18 with DM and 11 of 43 non-diabetic subjects (Table 1) were examined. Of the 11 non-diabetic subjects examined, 3 had impaired glucose tolerance (IGT) and 8 had normal glucose tolerance (NGT). All subjects have been confirmed positive for the 3243 mutation using methods previously described.[15]

In our five MIDD families, the mean age at onset of diabetes mellitus ($n = 18$) was 42.1 ± 13.8 years. In our retinopathy study subgroup ($n = 12$) the mean age at onset of DM was 42.3 ± 14.9 years (mean age of six subjects not examined 43.5 ± 10.4 years, $t_{16} = 0.18$, NS); all except one subject had diabetes onset before age 55 years. Of the 12 subjects with DM examined, 10 are now treated with insulin (83 %) although 4 of the 10 (H.II.1, H.II.9, J.II.1, W.II.2) were initially treated with oral therapy. Mean duration of diabetes in the 12 subjects was 17.0 ± 6.9 (range 5–26) years. All subjects had measurable C-peptide secretion with a mean fasting C-peptide of 0.46 ± 0.29 nmol l^{-1} (concurrent blood glucose 9.8 ± 3.8 mmol l^{-1}). There was no history of ketoacidosis in any of the subjects studied. MIDD subjects were therefore classified as having Type 2 diabetes mellitus.

The mean age of subjects with diabetes, at first retinal examination, was higher than those without diabetes (respectively, 54.1 ± 12.2 yr and 38.3 ± 20.5, $p = 0.03$, $t_{21} = 1.72$). All study subjects were examined by an ophthalmologist (PM). Corrected visual acuity was recorded and a retinal examination conducted including slit lamp biomicroscopy and retinal photography of seven standard fields through dilated pupils. Pigmentary retinopathy was defined as being present or absent on retinal examination according to diagnostic features previously described.[6,16] Retinal photographs were used to assess diabetic retinopathy and scaled using the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) methods.[17] Fluorescein angiography was performed in 11 of 12 DM subjects, 2 of 3 IGT subjects, and 3 of 8 NGT subjects.

In all subjects with the mutation (diabetic and non-diabetic), blood was collected between 08.30 and 09.30, after an overnight fast, for assay of RBC sorbitol, glucose, lactate, and pyruvate. Subjects with diabetes mellitus also had blood taken for glycosylated haemoglobin (HbA_{1c}). Red blood cell sorbitol, glucose, and HbA_{1c} were also collected, after an overnight fast, from 12 randomly selected Type 2 DM subjects from the general diabetes clinic at Westmead Hospital, all of whom tested negative for the 3243 mtDNA mutation and who were matched for age to the subjects with MIDD. Ten non-diabetic control subjects, matched for age to non-diabetic subjects with the 3243 mutation, had blood taken for assay of glucose and RBC sorbitol after having fasted for 4 h.

Table 1. Retinal findings in all subjects examined with the 3243 mutation

MIDD subjects	Current age	Age DM diagnosed	DR	PRet yes/no	Age PRet examination	Non-diabetic Subjects	Glucose tolerance	PRet yes/no	Age PRet examination
H.II.1-M[a]	72	50	absent	yes	62	R.III.9-M	IGT	no	77
H.II.7-M	71	54	NPDR	yes	65	O.III.2-F	IGT	yes	57
H.II.9-M	66	45	absent	yes	51	O.IV.6-F	IGT	yes	17
H.II.12-M	58	39	absent	yes	58	R.IV.4-F	NGT	yes	46
H.III.3-M	43	21	absent	yes	43	R.IV.6-F	NGT	no	38
O.III.1-M	66	47	NPDR	yes	56	R.V.2-F	NGT	no	40
R.III.8-M	82	76	absent	no	81	R.VI.3-M	NGT	no	15
R.IV.3-F[a]	47	26	NPDR	yes	47	W.III.5-M	NGT	no	17
R.V.1-M	46	41	absent	yes	43	H.IV.1-F	NGT	yes	31
J.I.1-F	74	48	absent	yes	61	O.II.7-F	NGT	no	61
J.II.1-F	48	30	absent	yes	46	O.IV.7-M	NGT	no	22
W.II.2-F	38	30	absent	yes	37				

[a]Subject now deceased
M, male; F, female; DR, diabetic retinopathy; PRet, pigmentary retinopathy; NPDR, non-proliferative diabetic retinopathy; IGT, impaired glucose tolerance, NGT, normal glucose tolerance. Current age is age at most recent examination for DR.

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Analytical Methods

Red blood cell sorbitol was measured using an enzymatic colorimetric method (D-Sorbitol kit, Boehringer Mannheim, Australia). In brief, 3 ml whole blood was collected onto 3 ml ice-cold 6 % perchloric acid and centrifuged at 3000 rev min^{-1} for 15 min within 1 h of collection. Supernatant was removed and brought to pH 8 using 2N KOH, allowed to stand on ice for 15 min and then filtered to remove perchlorate precipitate. Protein-free filtrate, 0.5 ml, was added to a reaction mixture which consisted of 0.5 ml distilled water, 0.3 ml potassium phosphate/triethanolamine buffer, pH 8.6/ triton X-100, 1.12 mg nicotinamide-adenine dinucleotide (NAD), 0.16 U diaphorase and iodonitrotetrazolium chloride solution. The iodonitrotetrazolium chloride is reduced by NADH (produced in the presence of sorbitol dehydrogenase) to a formazan in the presence of diaphorase which is measured at its maximum at 492 m wavelength. Sorbitol dehydrogenase, 0.042 U, was added to the reaction mixture and change in absorbance was determined when substrate exhaustion was complete (25 min).

Plasma glucose was measured using a glucose oxidase method (Yellow Springs Instruments, YSI 2300), pyruvate was measured using a spectrophotometric method ('Pyr', Boehringer Mannheim), normal range 40–100 μmol l^{-1}, and lactate was measured using Kodak Ektachem Clinical Chemist slides ('LAC'), normal range 0.63–2.44 mmol l^{-1}. Glycosylated haemoglobin was measured using high performance liquid chromatography, normal range $<$ 6 %.

Statistical Analysis

All data are expressed as means $\pm$ standard deviation (SD). Categorical variables were compared using chi-square. Continuous variables were compared using the Student *t*-test. Group means for sorbitol values were compared using ANOVA. Simple regression was used to analyse glucose and sorbitol relationships and logistic regression ('SPIDA' v.6, 1992, Statistical Computing Laboratory Pty Ltd, Australia) was used to analyse associations between the outcome variable, pigmentary retinopathy, and the explanatory variables (glucose tolerance and age). Statistical significance was considered at the 5 % level for all analyses. Odds ratios (OR) and 95 % confidence intervals (CI) are presented.

Results

Diabetic Eye Disease

Diabetic retinal changes were absent in 9 of 12 diabetic subjects (75 %) and in all subjects with normal or impaired glucose tolerance. Three (25 %) were classified as having mild non-proliferative diabetic retinopathy on retinal photographs, which was confirmed on fluorescein angiography in two. In detail, subject R.IV.3 had evidence of previous macular laser therapy (the indication for this was not determined) but had no current evidence of any diabetic retinopathy after 21 years of DM. Subject O.III.1 had microaneurysms only (level 20, WESDR scale) and H.II.7 had microaneurysms and haemorrhages (level 30, WESDR scale). Both had DM duration of 17 years. No MIDD subject had evidence of more severe retinopathic lesions such as cotton wool spots, intra-retinal microvascular abnormalities, venous beading or retinal new vessels. Of all subjects examined (diabetic and non-diabetic, $n = 23$), none had signs of cortical or posterior subcapsular cataract. Mean HbA_{1c} in diabetic subjects was 9.0 $\pm$ 1.2 (range 6.9–11.3) % and 9 of the 12 had $HbA_{1c} > 8$ % consistent with suboptimal glycemic control.

Pigmentary Retinopathy

Corrected visual acuities varied from normal (6/6) to severely impaired (6/60). Corrected visual acuity was normal in 8 of 12 diabetic subjects and 9 of 11 non-diabetic subjects. Pigmentary retinopathy was found in 15 of the 23 subjects (65 %, Table 1): 11/12 DM subjects, 2/3 IGT subjects, and 2/8 with NGT. The pigmentary retinopathy observed in all patients has previously been described[14] and could be detected from clinical appearance prior to fluorescein angiography.

Interaction of Pigmentary Retinopathy, Diabetes, and Age

There was no significant difference in mean age between subjects with (49.4 $\pm$ 14.6 (range 17–72) years) or without pigmentary retinopathy (43.6 $\pm$ 25.9 (range 15–79) years; $t_{21} = 0.69$, $p = 0.5$). In a logistic regression model to determine the relative effects of abnormal glucose tolerance and age on development of pigmentary retinopathy there was no association between pigmentary retinopathy and increasing age, OR 0.94 (CI 0.86, 1.03, $p = 0.6$). However, compared to subjects with NGT, abnormal glucose tolerance was significantly associated with PRet, OR 19.5 (CI 2, 173; $p = 0.008$). Also, as glucose tolerance changed from normal to impaired to diabetic range, there was a statistically significant increase in the odds of developing pigmentary retinopathy (OR 11.8, 95 % CI 1.5–94.0, $p = 0.02$). The disparity in age between subjects with normal, impaired and diabetic glucose tolerance meant that an adjustment for age could not be made and is therefore a potential confounder.

Red Blood Cell Sorbitol, Lactate, and Pyruvate Results

There was no significant difference in mean age between diabetic subjects with and without the 3243 mutation (mean age, respectively, 59.3 $\pm$ 14.5 and 63.7 $\pm$ 13.0

years, NS) or between non-diabetic subjects with and without the 3243 mutation (mean age, respectively, 40.6 ± 21.4 and 38.9 ± 9.1 years, NS). There was a significant difference in mean red blood cell sorbitol levels between the four groups ($F_{3,36} = 2.82$, $p = 0.05$, ANOVA): diabetic and non-diabetic subjects with the 3243 mutation and diabetic and non-diabetic controls (values, respectively, 1.18 ± 0.2, 1.13 ± 0.5, 1.72 ± 1.1 and 1.0 ± 0.4, × 10^{-2} g l^{-1}, × 10^{-2} g l^{-1}). There was, however, no difference in mean red blood cell sorbitol concentrations between MIDD subjects and diabetic controls ($p = 0.13$) and there was no difference in mean red blood cell sorbitol between MIDD subjects and non-diabetic subjects with the 3243 mutation ($p = 0.81$) despite a significant difference in ambient mean blood glucose concentration between the latter two groups (mean fasting blood glucose respectively, 11.7 ± 5.2 and 5.0 ± 0.6 mmol l^{-1}, $t_{15} = 3.62$, $p = 0.002$).

Previous studies[10,11,27] have demonstrated a significant positive linear relationship between ambient blood glucose and red blood cell sorbitol concentrations, both *in vivo* and *in vitro*. Intact human red cells are capable of converting glucose to sorbitol and the intracellular sorbitol is a reflection of the surrounding glucose concentration and flux through the polyol pathway. As we postulated diabetic subjects with a mitochondrial mutation were likely to have reduced polyol pathway activity, adjustment for ambient blood glucose was made to see if there were differences apparent in polyol pathway activity between the groups. When red blood cell sorbitol levels were adjusted for ambient blood glucose concentration, the adjusted sorbitol levels were significantly lower in MIDD subjects than in the diabetic control group (respectively, 1.16 ± 0.5 and 2.03 ± 1.1, × 10^{-3} g $mmol^{-1}$, $t_{18} = 2.19$, $p = 0.04$), indicating reduced sorbitol accumulation in subjects with MIDD. Adjusted sorbitol levels in MIDD subjects were also significantly lower than in non-diabetic subjects with the 3243 mutation (2.3 ± 0.1 × 10^{-3} g $mmol^{-1}$, $t_{15} = -2.91$, $p = 0.01$) and control subjects (2.0 ± 0.8^3 g $mmol^{-1}$, $t_{17} = -2.86$, $p = 0.01$).

To explore further the relationship between ambient blood glucose and sorbitol concentration, regression analysis was performed. A positive linear relationship was observed between ambient blood glucose and RBC sorbitol level in diabetic and non-diabetic control subjects ($R = 0.45$, $p = 0.03$) but not in diabetic and non-diabetic subjects with the 3243 mutation ($R = 0.11$, $p = 0.67$; Figure 1). When the diabetic control subject with sorbitol concentration of 0.048 g l^{-1} was removed from the former regression analysis, a significant relationship was no longer observed ($p = 0.25$).

Pyruvate levels were significantly higher in subjects with MIDD compared with non-diabetic subjects with the 3243 mutation (respectively 101.8 ± 40.7 and 66.5 ± 20.5 μmol l^{-1} $t_{15} = 2.21$, $p = 0.04$). There was, however, no difference in mean lactate concentration between MIDD subjects and non-diabetic subjects with the 3243 mutation (respectively, 2.18 ± 0.56, 1.77 ± 0.85 mmol l^{-1}, $t_{15} = 1.19$, $p = 0.25$).

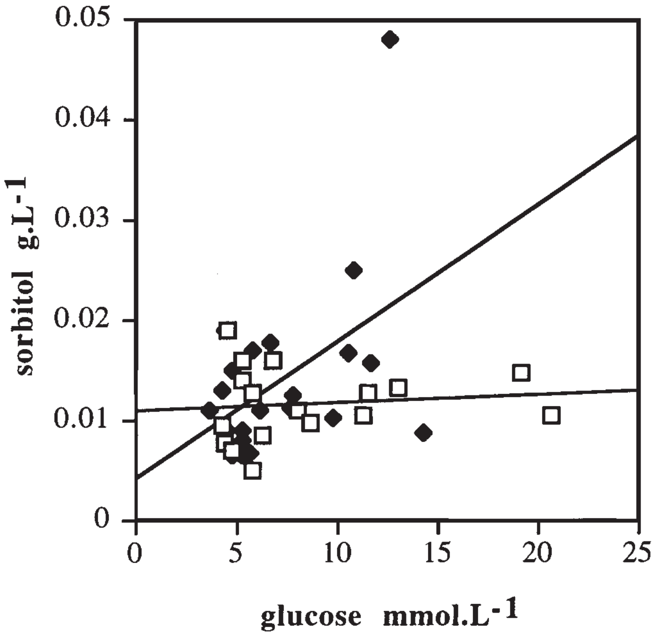

Figure 1. Red blood cell sorbitol and plasma glucose concentration in control diabetic and non-diabetic subjects (◆) and diabetic and non-diabetic subjects with the 3243 mutation (□). A significant positive linear relationship was found between red blood cell sorbitol and glucose in the control group ($R^2 = 0.21$, $p = 0.03$) but no relationship in the subjects with the 3243 mutation ($R^2 = 0.01$, $p = 0.67$)

Discussion

Diabetic retinopathy appears to have a reduced prevalence (25 %) and severity in MIDD, despite the presence of a number of risk factors for the development of diabetic retinopathy such as a long mean duration of diabetes (17.0 years), poor glycaemic control (mean HbA_{1c} 9 %) and a high prevalence of insulin therapy (83 %). Diabetic retinopathy, when present, was mild, rating a maximum of level 30 on the WESDR scale. Red blood cell sorbitol levels adjusted for ambient blood glucose concentration, were reduced in subjects with MIDD compared with a diabetic control population without the mitochondrial mutation, supporting our hypothesis that the 3243 mutation reduces mitochondrial ATP and free NAD^+ generation resulting in decreased glucose metabolism by the polyol pathway. Additionally, we have identified a strong positive association between abnormal glucose tolerance and pigmentary retinopathy (OR 19.5) in subjects with the 3243 mutation but we did not find any association between increasing age and pigmentary retinopathy.

We propose two possible explanations for our findings. Firstly, the metabolic derangements which are a consequence of the 3243 mutation may contribute both to protection from diabetic retinopathy and increase in pigmentary retinopathy in the retina. Secondly, the

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pigmentary retinopathy itself, through loss of RPE cells may act in a manner similar to retinal photocoagulation to prevent the development of significant diabetic retinopathy. This genetic model of diabetes may prove useful in understanding the pathogenesis of diabetic eye disease and other diabetes complications.

The prevalence of any diabetic retinopathy (25 %) and absence of proliferative diabetic retinopathy in our subjects is lower than expected given the reported prevalence of diabetic retinopathy in a large Australian diabetic population of 5519 Type 1 and Type 2 DM subjects.[18] The Australian reference diabetic population was of similar socioeconomic status to the present study population and consisted of a mixed population of Type 1 and Type 2 subjects. Four of the study subjects came from the same geographic region as the reference population. After diabetes duration of 15–19 years the prevalence of any diabetic retinopathy in the reference population was 69–88 % and the prevalence of proliferative diabetic retinopathy years was 9–20 %. The Australian prevalence data are similar to data previously reported in the Wisconsin study.[19]

The present study supports the findings of others[1,7] who found a low prevalence of diabetic retinopathy in subjects with MIDD. Vialettes *et al.*[1] reported an absence of diabetic retinopathy in four subjects with MIDD and durations of diabetes varying from 9 to 35 years. All subjects also had pigmentary retinopathy. Katagiri *et al.*[7] studied 17 subjects with MIDD and reported 1 with blindness due to diabetic retinopathy but no other cases of diabetic retinopathy. The mean duration of diabetes in the 17 subjects was 8.4 ± 5.6 years, less than the present study. 'Retinitis pigmentosa' was apparently absent in all subjects in the study of Katagiri *et al.*, however, the method of retinal assessment was not detailed.

Two other studies have found diabetic retinopathy in subjects with MIDD due to the 3243 mutation[8,9]. Suzuki *et al.*,[8] identified diabetic retinopathy in 7/14 MIDD subjects, 6 of whom had NPDR. The presence or absence of pigmentary retinopathy was not determined. The mean duration of diabetes, in the study of Suzuki *et al.* was 7.3 years. Kishimoto *et al.*,[9] found 'retinopathy' in 4 of 6 MIDD subjects with mean duration of diabetes of 18.7 years. We could find no previous reports of cataract in subjects with MIDD.

Glucose disposal by the sorbitol pathway has been proposed as a potential mechanism for the development of diabetic retinopathy.[20,21] The 3243 mutation may alter the normal pathways of glucose metabolism through reduced ATP production by the electron transport chain[22] and reduced generation of free NAD^+. NAD^+ is produced as a by-product of the electron transport chain and is a necessary cofactor for many enzymes both in the glycolytic and sorbitol dehydrogenase pathway (Figure 2). The combined reduction in ATP and NAD^+ availability means that NAD^+ generated from the conversion of pyruvate to lactate, rather than being utilized to catalyse

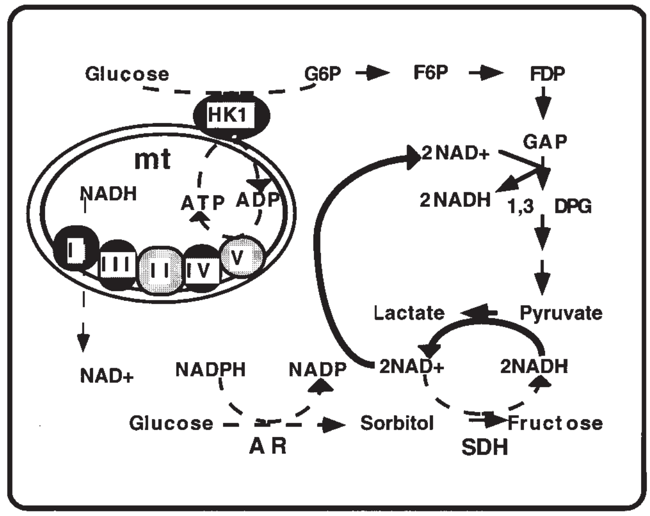

Figure 2. Alterations in the interaction between the glycolytic and polyol pathways and the mitochondrial electron transport chain due to the presence of a mitochondrial genome mutation: NAD+ generated from the conversion of pyruvate to lactate, rather than being utilized to convert sorbitol to fructose by sorbitol dehydrogenase (SDH), is diverted to the glycolytic pathway to maintain availability of ATP for glycolysis; mt, mitochondrion; AR, aldose reductase

the conversion of sorbitol to fructose by sorbitol dehydrogenase, may be preferentially diverted to the glycolytic pathway to maintain availability of ATP from glycolysis in a situation akin to hypoxia. Glucose disposal via the polyol pathway was apparently reduced in MIDD subjects despite the presence of hyperglycaemia, and this could explain the decreased prevalence of diabetic eye disease in subjects with MIDD. The absence of difference in sorbitol levels adjusted for ambient blood glucose in non-diabetic subjects with the 3243 mutation compared with MIDD subjects and diabetic and non-diabetic controls suggests mitochondrial function was less impaired than in subjects with MIDD.

Our second finding was an increased expression of pigmentary retinopathy in subjects with abnormal glucose tolerance and the 3243 mutation (OR 19.5). While increasing age did not appear to have a direct effect on the development of pigmentary retinopathy, an effect of age on glucose tolerance could not be excluded. In support of an effect of abnormal GT on pigmentary retinopathy expression, a number of studies have previously reported macular pigmentary abnormalities in people with DM and impaired glucose tolerance.[23,24] We hypothesize that hyperglycaemia in conjunction with the 3243 mutation will lead to higher lactate levels in the RPE, as increased lactate production has been demonstrated in the RPE of diabetic animals.[21] Reduced ATP availability and increased cellular lactate within the highly metabolically active RPE,[26] may contribute to RPE damage and contribute to the greater expression of pigmentary retinopathy in subjects with diabetes and the 3243 mutation. Although a systemic increase in lactate[25] was not demonstrated in the present study, in diabetic

subjects with the 3243 mutation, a systemic increase in pyruvate was observed. Abnormal pyruvate metabolism in subjects with the 3243 mutation has previously been demonstrated to result in intracellular acidosis using ^{31}P-NMR spectroscopy of skeletal muscle following exercise.[1] Increased blood lactate concentrations may have been masked in the present study by hepatic recycling of lactate via the Cori cycle in the fasting state[1] and lactate responses following a glucose challenge or exercise may have given a better indication of the potential for lactate accumulation at the tissue level. An alternative explanation for the coexistence of abnormal glucose tolerance and pigmentary retinopathy may be that subjects with MIDD have a greater proportion of mutant mtDNA in all tissues and therefore greater penetrance of all clinical features of the 3243 mutation.

Finally, the reduced severity of diabetic retinopathy may be due to the pigmentary retinopathy *per se*. Pigmentary retinopathy may reflect widespread damage to the RPE which could prevent development of diabetic retinopathy in a manner akin to retinal photocoagulation. In support of this thesis, Katagiri *et al.*[7] identified one diabetic subject with the 3243 mutation who had severe proliferative diabetic retinopathy but did not have 'retinitis pigmentosa'. Further confirmation requires more extensive identification of MIDD subjects without pigmentary retinopathy to be assessed for severity of diabetic retinopathy.

In conclusion, although the numbers studied are small, our results suggest reduced metabolism of glucose via the polyol pathway in patients with mitochondrial genome mutations may be responsible for the decreased prevalence of diabetic retinopathy and cataract. While pigmentary retinopathy does occur in the absence of diabetes, abnormal glucose tolerance is positively associated with pigmentary retinopathy, suggesting it may contribute to RPE degeneration. An interaction between the two retinopathies may also explain the decrease in prevalence of diabetic retinopathy. Further studies of polyol pathway activity are warranted in subjects with MIDD. Confirmation of the metabolic effect of mitochondrial DNA mutations on RPE cells '*in vitro*' would further help to clarify our findings. MIDD is a natural model for assessing the impact of reduced ATP and free NAD^+ on cellular function and potentially leads us to a greater understanding of the pathogenesis of diabetic eye disease and other diabetes complications.

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